OPEN ACCESS

African Journal of
Pharmacy and Pharmacology

8 June, 2018
ISSN 1996-0816
DOI: 10.5897/AJPP
www.academicjournals.org

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African Journal of Pharmacy and Pharmacology

Table of Contents: Volume 12 Number 19 8 June, 2018

ARTICLES

Vol. 12(19), pp. 231-239, 8 June, 2018
DOI: 10.5897/AJPP2018.4917
Article Number: 3FAFF4E57336
ISSN: 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Anti-hyperglycaemic and anti-hyperlipidemic effect of aqueous leaf extract of *Vernonia amygdalina* in Wistar rats

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Received 28 March, 2018; Accepted 14 May, 2018

Diabetes mellitus is a complex metabolic disorder characterised by impaired glucose tolerance and hyperglycemia, which is caused by either lack of or resistance to insulin in tissues; this disease causes significant morbidity and mortality largely due to its end–organ complications. Traditional treatment of diabetes mellitus has involved the use of several plant extracts; in this study the efficacy of aqueous leaf extract of *Vernonia amygdalina* (VA) was sought as a treatment module for alloxan induced diabetic rats. The aqueous leaf extract of VA was administered in three (3) different doses (40, 80 and 120 mg/kg) to non-diabetic and alloxan-induced diabetic rats. The body weights of the experimental animals were taken along with blood samples collection at baseline, on 7th, 14th and 21st days, thereafter the blood glucose and serum lipid levels were determined. The aqueous leaf extract of VA showed statistical significant ($p< 0.05$) reduction of blood glucose, serum triglyceride and cholesterol, as well as body weight in both non-diabetic and alloxan-induced diabetic rats. This study showed that aqueous leaf extract of VA administered at different doses contains anti-hyperglycemic and lipid lowering activities, with 80 mg/kg body weight dosage appearing to be the minimum effective dose; suggesting that aqueous leaf extract of VA is likely to contain actives that could be important in the control of blood glucose and serum lipid levels in diabetics.

Key words: *Vernonia amygdalina*, diabetes mellitus, blood glucose, anti-hyperglycaemic, anti-hyperlipidemic.

INTRODUCTION

Diabetes mellitus (DM) is a disease caused by either lack of insulin secretion or decreased sensitivity of tissues to insulin in which glucose metabolism is impaired (Saltiel and Kahn, 2001). This disease has been reckoned as one of leading health problems in Africa, which contributes significantly to morbidity and mortality (Garcia et al., 1974) and adversely affecting both quality and length of life. The prevalence of diabetes mellitus in

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Nigeria has been reported to have increased from 2.2% in 1997 to 5.0% by 2013 (Oputa and Chinenye, 2015), diabetes mellitus is amongst the leading cause of mortality in Africa (Hall et al., 2011). Unlike in Africa, diabetes mellitus prevalence in India has reached a pandemic level, with number of diabetic patients reaching over 62 million (Kaveeshwar and Cornwall, 2014) and reflectng the global burden of the disease. Various types of this disease have been identified (National Diabetes Data Group, 1979) and some prevention and treatment measures have been recommended (Oputa and Chinenye, 2015; Pan et al*.,* 1997).

The chronic hyperglycemia that results in this ailment is associated with many abnormalities in various organs of the body (Pignone et al*.,* 2009). Some studies have shown that lipid profile is also altered in diabetics (Nakhjavani et al., 2006), and this dyslipidemia predisposes the diabetic patients to cardiovascular complications (Barrett-Connor, 1983; Rader, 2007). Most diabetic patients especially in Nigeria are grossly faced with inadequate medicine, and cost of managing the disease is high. In addition, the use of available antidiabetic drugs like metformin have several side effects, which compounds the existing problems faced by health care–givers (Robertson, 1995). The rise in prevalence of diabetes mellitus has necessitated the need for development of adequate and sophisticated methods for its management and treatment to forestall the danger and health complications involved with the disease.

Vernonia amygdalina (VA) is a perennial shrub commonly known as bitter leaf, which belongs to the family of *Asteraceae* (Iwalokun et al., 2006). In ethnomedicine, *V. amygdalina* leaves are consumed either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for treatment of various illnesses (Igile et al., 1995). In North America, all the known 17 species of *Vernonia* have been shown to possess properties like blood purifier, uterus toner, and also ability to prevent atherosclerosis (Erasto et al., 2007; Nwanjo, 2005). In herbal medicinal practice, aqueous leaf extract of *V. amygdalina* is recommended for patients to treat anemia, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems. A number of experimental findings have presented *V. amygdalina* as possessing anti-pathogenic and other beneficial medicinal effects; for example, leaf extract of *V. amygdalina* has been shown to suppress, delay or kill cancer cells, possess anti-fungal, anti-plasmodia, anti-bacterial (Kupchan et al., 1969; Ijeh et al., 1996; Akinpelu, 1999; Jisaka et al., 1993); antioxidant (Torel et al., 1986; Igile et al., 1994; Iwalokun et al., 2006; Adaramoye et al., 2008; Owolabi et al., 2008), hepato and nephron–protective effects (Ijeh and Obidoa, 2004; Iwalokun et al., 2006). Bioactive peptide of aqueous leaf extract of *V. amygdalina* is a potent anti–cancer agent (Izevbigie, 2003; Izevbigie et al., 2004). Dietary incorporation of *V. amygdalina* has been reported to lower serum triacylglycerol and LDL level, normalize cholesterol concentration and concomitantly increased HDL (Ugwu et al., 2010; Nwanjo, 2005) while ethanolic leaf extract of *V. amygdalina* has been reported to keep the lipid profile of rats in normal range (Ekpo et al., 2007). Lowering of blood sugar has also been reported by Akah et al., (2004). The utilization of herbal extracts to treat diabetes related illness has therefore increased over the years (Rates, 2001). According to WHO (1993), due to poverty and lack of access to modern medicine, moderate percentage of world population found in the developing countries depend mostly on plants for primary health care. Considering the use of *V. amygdalina* leaves in ethnomedicine, this study further investigated experimentally, the capability of aqueous leaf extract of *V. amygdalina* in treating of alloxan–induced diabetic rats.

MATERIALS AND METHODS

Fresh leaves of *V. amygdalina* were purchased from Kenyatta Market in Uwani, Enugu State, Nigeria. They were identified and authenticated by Mr. Onyeukwu Chijioke of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium for future reference with number: (UNH7a).

Preparation of the extract

Preparation of the extract was done using the method of Akah et al. (1992). The leaves were washed and dried under shade for 7 days. It was pulverized into powder using electric blender. Five and half (5.5) liters of distilled water was added to 1200 g of the *V. amygdalina* leaf powder and boiled for 30 min under reflux at 80°C and then allowed to cool for 20 min. Thereafter, the mixture was filtered using Whatman No.1 filter paper. The filtrate was concentrated using water bath at a temperature of 50°C; then evaporated to dryness to give a dark green solid paste with a yield of 11.3%.

Phytochemical analysis

Alkaloid determination

To determine alkaloid content, 5 g of the sample was weighed into a 250-mL beaker and 200-mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one–quarter of its original volume. Concentrated NH_4OH was added drop–wise to the extract and the precipitate was collected and washed with dilute NH_4OH and then filtered. The alkaloid residue was dried and weighed (Harbone, 1973).

Flavonoid determination

To determine flavonoid content, 10 g of the sample was treated with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through a Whatman filter paper No. 42; the filtrate was later transferred into a crucible and evaporated to

dryness over a water bath; thereafter, it was weighed until constant weight was obtained (Boham and Kocipai–Abyazan, 1994).

Tannin determination

To determine tannin content, 500 mg of the sample was weighed into a 50-mL plastic bottle. 50 mL of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50-mL volumetric flask and made up to 50-mL with distilled water. 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.1 m $FeCl_3$ in 0.1 N HCl and 0.008 M $K_4[Fe(CN)_6]$ 3 H_2O. The absorbance was measured at 120 nm within 10 min (Van–Burden and Robinson, 1998).

Glycoside determination

The method of El–Olemy et al., (1994) was used. 1 g of the *V. amygdalina* powder was soaked in 10 mL of 70% alcohol for 2 h and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before addition of freshly prepared Baljet reagent (containing 95 mL aqueous 1% picric acid + 5 mL of 10% aqueous NaOH). The difference between the intensity of colors of the experimental and blank (distilled water and Baljet reagent sample gives the absorbance and is proportional to the concentration of the glycoside.

Saponin determination

The method used was that of Obadoni and Ochuko, (2001). 20 g of the *V. amygdalina* leaf powder was added into a conical flask and 100 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with 200 mL of 20% ethanol. The mixture of the extracts was reduced to 40 mL over water bath at 90°C. The concentrate was transferred into a 250-mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously; also, the aqueous layer was recovered while the ether discarded. The purification process was repeated and 60 mL of n–butanol was added. The combined n–butanol extracts were washed twice with 10 mL of 5% aqueous NaOH. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage weight.

Determination of the phenolic compound

To determine the phenolic compound, 10 g of the extract was weighed and dissolved in 100 mL of distilled water, with 1 mL of this solution transferred to a test tube; thereafter, 0.5 mL of the Folin–Ciocalteu reagent and 1.5 mL (20% of Na_2CO_3 solution) was added and the volume made up to 8 mL with distilled water followed by vigorous shaking and finally allowed to stand for 2 h after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from vigorous diluted concentrations of gallic acid (Kahkonen et al., 1999).

Experimental animals

Thirty-five (35) male albino Wistar rats weighing 175–285 g was used for the study. The rats were purchased from the Animal House of Department of Pharmacology, University of Nigeria Enugu Campus (UNEC) and were housed in a clean ventilated wire mesh cages, at room temperature. Here, food and water were given *ad libitum*, with 12 h dark and 12h light cycle also observed. Animals were used according to the animal welfare regulation of the institution.

Induction of diabetes

Diabetes was induced using the method of Imaga et al., (2013). The rats were fasted overnight at least 10 h after which, 10% alloxan solution diluted in normal saline was administered to the rats intraperitoneally; and were allowed access to food and water 30 min after alloxan administration. The rats were confirmed diabetic after 24 h with glucometer.

Animal grouping and administration of the extract

The rats were divided into 3 major groups (A, B and C) with groups A and C having sub–groups. The administration of the extract was done according the method of Akah et al., (1992).

Group A: The control group; was divided into 3 sub–groups (Ai, Aii and Aiii) of 5 rats per group.

Group Ai: Non–diabetic that received water only.

Group Aii: Untreated diabetic that was given water only.

Group Aiii: Diabetic that was treated with 5 mg/kg body weight of Glibenclamide (a standard anti–diabetic drug) orally.

Group B: Non–diabetic that received 80 mg/kg of aqueous leaf extract of *V. amygdalina*.

Group C: The diabetic group treated with different doses of aqueous leaf extract of VA. It was divided into 3 sub–groups: Ci, Cii, and Ciii of 5 rats per group.

Group Ci: received 40 mg/kg body weight of aqueous leaf extract of *V. amygdalina*.

Group Cii: received 80 mg/kg body weight of aqueous leaf extract of *V. amygdalina*.

Group Ciii: received 120 mg/kg body weight of the aqueous leaf extract of *V. amygdalina*.

Tween 80 was used as a vehicle for the delivery of the drug and the aqueous leaf extract of VA to the animals. The extract was administered orally for 21 days before sacrifice.

Collection of blood sample

Blood samples were collected from the media canthus of the eye by retro orbital puncture for serum preparation. Samples were taken at baseline, on Day 7, 14, and 21 and the following parameters determined: blood glucose and lipid; body weight of the animals was also measured on the days as samples were collected.

Blood glucose estimation

The blood glucose levels were estimated with the tail prick method using glucose oxidase–peroxidase reactive strips (Accu–check, Roche Diagnostic, USA). Thereafter, blood glucose was determined using glucometer.

Table 1. Percentage composition of detectable phytochemical constituent of aqueous leaf extract of VA.

S/N	Constituent	%
1	Alkaloid	4.717
2	Saponin	5.660
3	Glycoside	0.04
4	Flavonoid	0.084
5	Phenol	0.188
6	Tannin	1.703

Measurement of body weight

The weight of the rats were measured and recorded to the nearest gram (g) using the electronic weighing scale Model No.: LP505A made in China.

Estimation of serum triglyceride

It was done using the method of McGowan et al., (1983). 5 µL of the sample was pipetted into test tubes using micro–pipette. 1000 µL of triglyceride reagent was added. The mixture was incubated for 10 min at 25°C, and the absorbance of the samples and standard against blank was recorded using spectrophotometer.

Triglyceride concentration = Sample/Standard × Standard Concentration (mmol/L)

Estimation of serum cholesterol

Serum cholesterol level was determined by the method of Allain, (1974). 5 µL of serum was pipetted into a test tube. Thereafter, 500 µL of cholesterol reagent was then added, the mixture was incubated for 10 min at 25°C. The measurement of the absorbance of the sample and standard against reagent blank was done using spectrophotometer.

Concentration of Cholesterol = Sample/Standard × Concentration of Standard (mmol/L)

Estimation of high density lipoprotein (HDL)

HDL was determined using the method of Assmann, (1984). 500 µL of serum plus 500 µL diluted precipitant were pipetted into tubes and centrifuged for 10 min at 4000 rpm. The clear supernatant (100 µL) plus 1000 µL HDL reagent was mixed and incubated for 10 min at 25°C. The absorbance of the sample and standard supernatant was measured against the reagent blank using spectrophotometer.

Concentration of HDL Cholesterol Supernatant = Sample/Standard × Concentration of Standard (mmol/L)

Estimation of low density lipoprotein (LDL)

LDL cholesterol = Total cholesterol – Triglyceride/5 – HDL cholesterol (mmol/L).

Data analysis

Data were analyzed using SPSS version 20. Results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) with post–hoc Dunnette`s test was used to compare the difference between groups. The p values ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis

Quantitative screening and analysis of aqueous leaf extract of *V. amygdalina* revealed that it contains a high percentage of saponin and alkaloid, mild percentage of tannin and few percentages of phenol, flavonoid and glycoside (Table 1).

The anti–hyperglycemic effect of aqueous leaf extract of *V. amygdalina* was evidenced in most of the treated groups. There was a significant decrease in blood glucose level on day (7 and 21) of Group B (non–diabetic treated with 80 mg/kg of leaf extract of *V. amygdalina*) compared with Group Ai (non–diabetic without treatment) (Table 2). A decrease in blood glucose level was also observed in Group Cii (Diabetic treated with 80 mg/kg body weight of aqueous leaf extract of *V. amygdalina*) when compared with Groups Aii and Aiii on days (7, 14 and 21) (Table 2). Tannin has been reported to inhibit alpha–amylase, sucrose, as well as the action of SGLUT–1 of the intestinal brush border (Tiwari and Rao, 2002) and some other enzymes (Zheng et al., 2009; Oprea et al., 2008).

The general reduction in blood glucose level could be as a result of the combined effect of the anti–hyperglycemic and hypoglycemic effect of some of the phytochemicals constituents of aqueous leaf extract of *V. amygdalina.* The phytochemical constituents responsible for this effect is yet unknown (Akah et al., 2004; Akah and Okafor, 1992); however, Erasto et al., (2009) suggested that antidiabetic property of extract of *V. amygdalina* could be associated with its ability to enhance glucose utilization and uptake by muscles and liver cells cultures. Effective blood glucose control is the key to preventing or reversing diabetic complication and improving quality of life in patients with diabetes mellitus. Thus, sustained reduction in hyperglycemia will reduce risk of developing more vascular complications (Muniappan et al., 2004). Anti–hyperglycemic activity of the aqueous leaf extract of

Table 2. Mean±SEM of changes in blood glucose level (mg/dL) of the experimental animals.

Period	Ai	Aii	Aiii	B	Ci	Cii	Ciii
Day (0)	121	600	339	115	600	600	600
Day (7)	99	101	104	87	539	83	446
Day (14)	87	319	193	92	600	142	575
Day (21)	146	254	131	99	445	101	500
Mean	113.25	318.5	191.75	98.25	546	231.5	530.25
Std. error	12.99	104.37	52.50	6.10	36.61	123.45	35.21

Ai– Normal control (Non–diabetic without treatment); Aii– Diabetic without treatment; Aiii– Diabetic treated with drug (Glibenclamide); B– Non–diabetic treated with 80 mg/kg of VA; Ci– Diabetic treated with 40 mg/kg of VA; Cii– Diabetic treated with 80 mg/kg of VA; Ciii– Diabetic treated with 120 mg/kg of VA.

Table 3. Mean±SEM of changes in body weight (g) of the experimental animals.

Period	Ai	Aii	Aiii	B	Ci	Cii	Ciii
Day (0)	265.53	190.09	242.83	261.69	173.27	203.82	202.53
Day (7)	297	188.5	259	287	151	210	192.5
Day (14)	316.31	199.36	262.56	316.62	163.63	201.45	192.85
Day (21)	335.03	200.22	264.27	277.66	153.98	200.55	199.06
Mean	303.4675	194.5425	257.165	285.7425	160.47	203.955	196.735
Std. error	14.83859	3.05203	4.90278	11.54286	5.04667	2.13386	2.44976

Ai– Normal control (Non–diabetic without treatment); Aii– Diabetic without treatment; Aiii– Diabetic treated with drug (Glibenclamide); B– Non–diabetic treated with 80 mg/kg of VA; Ci– Diabetic treated with 40 mg/kg of VA; Cii– Diabetic treated with 80 mg/kg of VA; Ciii– Diabetic treated with 120 mg/kg of VA.

V. amygdalina in this study was more effective at 80 mg/kg body weight of the extract (Table 2). Mechanism of action of leaf extract of *V. amygdalina* is not clearly understood (Akah and Okafor, 1992); thus, a devoted study to understand its mechanism of action is required.

There was a significant decrease in body weight in non–diabetic treated with aqueous leaf extract of *V. amygdalina* (Group B) compared to non–diabetic without treatment (Group Ai) on Day 7 and 21 as shown in Table 3. The decrease in body weight of non–diabetic treated with *V. amygdalina* compared to non–diabetic without treatment could be as a result of decreased feed intake by the animals. Phytochemical tannin could be responsible for weight loss because it affects nutrient utilization.

Significant decrease in body weight was observed among diabetic treated with *V. amygdalina* groups (Ci, Cii and Ciii) compared to diabetic treated with the reference drug as shown in Table 3 on the $7^{th,}$ 14^{th} and 21^{st} days. In diabetes mellitus, the obligatory renal water loss combined with the hyperosmolarity tends to deplete intracellular water, triggering the osmoreceptor of the thirst centre on the brain and polydipsia which leads to increase in water intake. The catabolic effects then prevailed, resulting in weight loss (UK Prospective Diabetes Study Group (UKPDS), 1998).

Significant decrease in serum triglyceride was observed in Group Ci (diabetic treated with 40 mg/kg of aqueous leaf extract of *V. amygdalina*) and Cii (diabetic treated with 80 mg/kg body weight aqueous leaf extract of *V. amygdalina*) compared with diabetic without treatment (Group Aii) and diabetic treated with the reference drug (Table 4). Adequate treatment of diabetes dyslipidaemia through diet is critical in reducing risk and complications, and the role of medicinal plants in the treatment of diabetes is an emerging important therapeutic approach. Aqueous leaf extract of *V. amygdalina* has been reported to possess anti–hypertriglyceridemic and hypolipidemic effects in alloxan induced diabetic model (Aka et al., 2004). Improved glycemic control following *V. amygdalina* therapy has been shown to decrease VDL and total triglyceride levels (Huupponen et al., 1984).

There was no significant decrease in serum cholesterol level of non–diabetic treated with aqueous leaf extract of *V. amygdalina* (Group B) compared to non–diabetic without treatment (control group) (Group Ai) (Table 5). Significant decrease in serum cholesterol level was observed in diabetic rats treated with (40 mg/kg) of aqueous leaf extract of *V. amygdalina* (Group Ci) on Day 21 compared to the diabetic treated with the reference drug (5 mg/kg body weight of glibenclamide) and diabetic without treatment (Group Aii) (Table 5). The level of serum cholesterol has been reported to be reduced on

Table 4. Mean±SEM of changes in serum triglyceride level (Mmol/L) of the experimental animals.

Period	Ai	Aii	Aiii	B	Ci	Cii	Ciii
Day (0)	3.55	4.02	3.64	4.09	4.81	4.35	3.26
Day (7)	4.19	3.93	4.38	4.17	4.34	4.33	3.17
Day (14)	3.89	4.09	4.09	4.03	4.15	3.99	4.11
Day (21)	3.6	4.14	4.28	4.31	2.04	2.6	4.41
Mean	3.8075	4.045	4.0975	4.15	3.835	3.8175	3.7375
Std. error	0.14735	0.04436	0.1644	0.05962	0.61319	0.41486	0.30751

Ai– Normal control (Non–diabetic without treatment); Aii– Diabetic without treatment; Aiii– Diabetic treated with drug (Glibenclamide); B– Non–diabetic treated with 80 mg/kg of VA; Ci– Diabetic treated with 40 mg/kg of VA; Cii– Diabetic treated with 80 mg/kg of VA; Ciii– Diabetic treated with 120 mg/kg of VA.

Table 5. Mean±SEM of changes in serum cholesterol level (Mmol/L) of the experimental animals.

Period	Ai	Aii	Aiii	B	Ci	Cii	Ciii
Day (0)	9.88	9.49	7.73	9.18	7.28	9.36	8.52
Day (7)	9.85	9.54	9.71	8.25	8.3	9.11	6.5
Day (14)	5.81	6.16	6.18	6	6.04	6.2	6.27
Day (21)	9.31	10	10.11	9.1	5.31	10.09	10.11
Mean	8.71	8.80	8.43	8.13	6.73	8.69	7.85
Std. error	1.01	0.89	0.91	0.86	0.66	0.85	0.91

Ai– Normal control (Non–diabetic without treatment); Aii– Diabetic without treatment; Aiii– Diabetic treated with drug (Glibenclamide); B– Non–diabetic treated with 80 mg/kg of VA; Ci– Diabetic treated with 40 mg/kg of VA; Cii– Diabetic treated with 80 mg/kg of VA; Ciii– Diabetic treated with 120 mg/kg of VA.

Table 6. Mean±SEM of changes in serum HDL level (Mmol/L) of the experimental animals.

Period	Ai	Aii	Aiii	B	Ci	Cii	Ciii
Day (0)	4.16	4.92	3.1	2.93	4.17	0.84	1.87
Day (7)	4	4.05	4.2	4.21	3.74	4.36	3.73
Day (14)	4.6	4.69	3.53	3.41	0.99	0.52	1.95
Day (21)	5.14	2.7	4.52	4.71	5.12	5.1	1.95
Mean	4.48	4.09	3.84	3.82	3.51	2.71	2.38
Std. error	0.25	0.51	0.32	0.39	0.88	1.17	0.54

Mean±SEM of changes in serum high density lipoprotein (HDL) level (Mmol/L) of diabetic and non–diabetic male albino rats. Ai– Normal control (Non–diabetic without treatment); Aii– Diabetic without treatment; Aiii– Diabetic treated with drug (Glibenclamide); B– Non–diabetic treated with 80 mg/kg of VA; Ci– Diabetic treated with 40 mg/kg of VA; Cii– Diabetic treated with 80 mg/kg of VA; Ciii– Diabetic treated with 120 mg/kg of VA.

diabetic rats treated with aqueous leaf extract of *V. amygdalina* (Gonzalez and Fevery, 1992, Nwanjo, 2007). Excess LDL–cholesterol could be deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions (Adaramoye et al., 2008). Reduction in elevated level of cholesterol could improve renal and hepatic functions. An earlier report by Iwalokun et al., (2006) has shown hepatoprotective potentials of leaf extract of *V. amygdalina* in mice.

Significant increase in serum HDL on the 7th day of the experiment was observed in Group B compared to Group Ai (Table 6). Similar observation was made in serum high density lipoprotein (HDL) on 7th and 21st days in diabetic rats treated with 80 mg/kg of aqueous leaf extract of *V. amygdalina* (Group Cii) compared to diabetic treated with the reference drug and diabetic without treatment (Table 6). This observation is consistent with earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice (Iwalokun et al., 2006). In this study, the use of 80 mg/kg body weight dosage numerically increased the HDL–Cholesterol on 7th and 21st days of the experimental animals compared to diabetic treated with the reference drug and diabetic without treatment. One of the important risk factors for cardiovascular disease (CVD) includes a low–level HDL–cholesterol. The association between a low level of HDL–cholesterol

Table 7. Mean±SEM of changes in serum LDL level (Mmol/L) of the experimental animals.

Period	Ai	Aii	Aiii	B	Ci	Cii	Ciii
Day (0)	3.91	4.82	2.29	4.38	0.92	6.51	4.63
Day (7)	3.74	3.68	3.48	2.51	2.59	2.78	1.3
Day (14)	2.23	0.68	0.79	0.76	3.16	4.76	0.53
Day (21)	0.15	0.06	0.2	0.04	0.12	0.13	0.2
Mean	2.51	2.31	1.69	1.92	1.70	3.55	1.67
Std. error	0.87	1.15	0.74	0.97	0.71	1.37	1.01

Ai–Normal control (Non–diabetic without treatment); Aii–Diabetic without treatment; Aiii–Diabetic treated with drug (Glibenclamide); B–Non–diabetic treated with 80 mg/kg of VA; Ci–Diabetic treated with 40 mg/kg of VA; Cii–Diabetic treated with 80 mg/kg of VA; Ciii–Diabetic treated with 120 mg/kg of VA.

and an increased risk of CVD has been established through epidemiological and clinical studies (Assmann and Gotto, 2004).

The protective roles of HDL cholesterol from CVD have been suggested to occur in various ways (Nofer et al., 2002). HDL exerts part of its anti–atherogenic effect by counteracting LDL oxidation and recent studies also showed that HDL promotes the reverse cholesterol transport pathway by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL (Yokozawa et al., 2006). Furthermore, the aqueous leaf extract of VA may probably have played the anti–atherogenic role through the elevation of HDL cholesterol.

Significant reduction of serum LDL was observed in Group B (non–diabetic treated with 80 mg/kg body weight of aqueous leaf extract of *V. amygdalina*) when compared to Group Ai (non–diabetic without treatment) on Day 7 of the experiment (Table 7).

There was also significant decreases in serum (LDL) level of diabetic treated with *V. amygdalina* (Group Ci to Ciii) compared to diabetic treated with the reference drug (Group Aiii) and diabetic without treatment (Group Aii) on 7^{th} day (Table 7). Plasma LDL–cholesterol level may be used in monitoring the treatment of patients with elevated blood cholesterol levels (Adaramoye et al., 2008). This result is in line with the finding of Imafidon and Okunrobo, (2012) where they observed that *V. amygdalina* extract reduced total blood cholesterol revealing a hypocholesterolemic tendency. It is now widely believed that an important signal for insulin secretion may be the link between glucose and lipid metabolism; and long–term exposure of islet cells to high levels of fatty acids result in β–cell dysfunction (lipotoxicity) (Krolewski et al., 1994).

Conclusion

Aqueous leaf extract of *V. amygdalina* possesses hypoglycemic, anti–hyperglycemic and lipid lowering activities, with 80 mg/kg body weight dosage showing the most potent dose at which aqueous leaf extract of *V. amygdalina* demonstrated highest activity. Since dyslipidaemia occurs in most diabetic patients, the utilization of lipid–lowering agents is now advocated for diabetic treatment and the findings from this study suggest that aqueous leaf extract of *V. amygdalina* could also be useful in this regard complementing its blood glucose lowering capacity. The results, therefore, justify the ethnomedicinal use of *V. amygdalina* leaves in treatment of diabetes mellitus, though further work is required to optimize the extract for extrapolation to humans, and understand its mechanism of action in enhancing positive effect in diabetes mellitus treatment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adaramoye OA, Akintayo O, Achem J, Fafunso MA (2008). Lipid lowering effects of methanolic extracts of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. Vascular health and risk management, 4:236-241.

Akah P, Njoku O, Nwanguma A, Akunyili D (2004). Effects of aqueous leaf extract of *Vernonia amygdalina* on blood glucose and triglyceride levels of alloxan–induced diabetic rats (*Rattus rattus*). Animal Research International,1(2):90-94.

Akah PA, Okafor CL (1992). Blood sugar lowering effect of *Vernonia amygdalina* Del, in an experimental rabbit model. Phytotherapy Research, 6(3):171-173.

Akinpelu DA (1999). Antimicrobial activity of *Vernonia amygdalina* leaves. Fitoterapia, 70:432-434.

Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC (1974). Enzymatic determination of total serum cholesterol. Clinical chemistry, 20(4):470-475.

Assmann G, Gotto AM (2004). HDL cholesterol and protective factors in atherosclerosis. Circulation, 109(23 suppl 1):III-8.

Assmann G, Jabs HU, Kohnert U, Nolte W, Schriewer H (1984). LDL–cholesterol determination in blood serum following precipitation of LDL with polyvinylsulfate Clinica Chimica Acta, 140(1):77-83.

Barrett–Connor E, Wingard DL (1983). Sex differential in ischemic heart disease mortality in diabetics: a prospective population–based study. American Journal of Epidemiology, 118(4):489-496.

Boham BA, Kocipai–Abyazan R (1994). Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pacific Science, 48:458-463.

Ekpo A, Eseyin AO, Ikpeme AO, Edoho EJ (2007). Some studies on some biochemical effects of *Vernonia amygdalina* amygdalina in rats. Asian Journal of Biochemistry, 2(3):193-197.
EL–Olemy MM, AL–Muhtadi FJ, AFIFI AFA (1994). Experimental Phytochemical. A laboratory manual. King Saud University Press, Saudi Arabia, pp. 21-27.
Erasto P, Grierson DS, Afolayan AJ (2007). Evaluation of antioxidant activity and the fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa. Food chemistry, 104(2):636-642.
Erasto P, Venter M, Roux S, Grierson DS, Afolayan AJ (2009). Effect of extracts of *Vernonia amygdalina* on glucose utilization of chang–liver, C2C12 muscle and 3T3–L1 cells. Pharmaceutical Biology, 47:175-181. Muniappan L, Leelavinothan P, Sandhya S, Ramesh B (2004). Insulin –secreagogue activity and cytoprotective role of the traditional anti–diabetic plant scoparia dulcis (sweet Broomweed). Life sciences, 23(7):75-77.
Garcia MJ, McNamara PM, Gordon T, Kannell WB (1974) Morbidity and mortality in diabetics in the Framingham population: sixteen year follow–up study. Diabetes, 23:105-111.
Gonzalez J, Fevery J (1992). Spontaneously diabetic biobreeding rats and impairment of bile and independent bile flow and increased biliary bilirubin, calcium and lipid secretion. Hepatology, 16:426-432,
Hall V, Thomsen RW, Henriksen O, Lohse N (2011). Diabetes in Sub Saharan Africa 1999-2011: epidemiology and public health implications. A systematic review. BMC Public Health, 11(1):564-565.
Harbone J (1973). "Phytochemical methods. Chapman and Hall, Ltd London, 21:49-188.
Huupponen RK, Viikari JS, Saarima H (1984). Correlations of serum lipids with diabetes control in sulphonyhurea treated diabetic patients. Diabetes Care, 7:575-578.
Igile GO, Wieslaw O, Burda S, Jurzysta M (1995). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. Journal of Agricultural and Food Chemistry, 43:2162-2166.
Igile GO, Wieslaw O, Marian J, Stanislaw B, Michael F, Adetunde F (1994). Flavonoids from *Vernonia amygdalina* and their antioxidant activities. Journal of Agricultural and Food Chemistry, 42:2445-2448.
Ijeh II, Nwugo VO, Obidoa O (1996). Comparative studies on the nutritive, phytochemical and antimicrobial properties of two varieties of *Vernonia amygdalina*. Plant Production Res. Comm. 1:71-75.
Ijeh II, Obidoa O (2004). Effect of dietary incorporation of *Vernonia amygdalina* Del. on AFB1–induced hepatotoxicity in weanling albino rats. Jamaican Journal of Science and Technology, 15:32-36.
Imafidon EK, Okunrobo OL (2012). Effects of *Vernonia amygdalina* Del. Extract on Cholesterol Level and Lipid Peroxidation Status in Rats Given Red Dye Adulterated Palm Oil Diets. British Journal of Pharmaceutical Research, 2(2):98-107.
Imaga NOA, Bamigbetan DO (2013). *In vivo* biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter leaf). International Journal of Nutrition and Metabolism, 5(2):22-27.
Iwalokun BA, Efedede BU, Alabi–Sofunde JA, Oduala T, Magbagveola OA, Akinwande AI (2006). Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen–induced hepatic damagein mice. Journal of medicinal food, 9(4):524-530.
Izevbigie EA, Bryant JL, Walker A (2004). A novel natural inhibitor of extracellular signal–regulated kinases and human breast cancer cell growth. Experimental Biology and Medicine, 229(2):163-169.
Izevbigie EB (2003). Discovery of water–soluble anticancer agents (edotides) from a vegetable found in Benin City, Experimental Biology and Medicine, 228:293-298.
Jisaka M, Ohigashi H, Takegawa K, Huffman MA, Koshmizu K (1993). Antinutritional and antimicrobial activities of bitter sesquiterpene lactones of *Vernonia amygdalina* a possible medicinal plant used by wild chimpanzee. Bioscience biotechnology and biochemistry, 57:833-834.
Kahkonen M.P, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry, 47(10):3954-3962.
Kaveeshwar SA, Cornwall J (2014). The current state of diabetes mellitus in India. The Australasian Medical Journal, 7(1):45
Krolewski AS, Warram JH, Christlieb AR (1994). Hyper–cholesterolemia: a determinant of renal function and death in IDDM patients with nephropathy. Kidney International Supplement, 45:125-131.
Kupchan SM, Hemingway RJ, karim A, Wermer D (1969). Tumour inhibitors XLVII vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. The Journal of organic chemistry, 34:3908-3911.
McGowan MW, Artiss JD, Strandbergh DR, Zak B (1983). A peroxidase–coupled method for the colorimetric determination of serum triglycerides. Clinical chemistry, 29(3):538-542.
Nakhjavani M, Esteghamati A, Esfahanian F, Heshmat AR (2006). Dyslipidemia in type 2 diabetes mellitus: more atherogenic lipid profile in women. Acta Medica Iranica, 44(2): 111-118.
National Diabetes Data Group, (1979). Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes. 28(12):1039-057.
Nofer JR, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A (2002). HDL and arteriosclerosis: eyond reverse cholesterol transport. Atherosclerosis, 161(1):1-16.
Nwanjo HU (2005). Efficacy of aqueous leaf extract of *vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. Nigerian Journal of Physiological Sciences, 20 (1–2):39-42.
Obadoni BO, Ochuko PO (2001). Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of pure and applied sciences, 8:203-208.
Oprea E, Radulescu V, Balotescu C, Lazar V, Bucur M, Mladin P, Farcasanu IC (2008) Chemical and biological studies of *Ribes nigrum* L. buds essential oil. Biofactors, 34:3-12.
Oputa RN, Chinenye, S (2015). Diabetes in Nigeria–a translational medicine approach. African Journal of Diabetes Medicine, 23(1):7-10.
Owolabi MA, Jaja SI, Oyekanmi OO, Olatunji OJ (2008). Evaluation of the antioxidant activity and lipid peroxidation of the leaves of *Vernonia amygdalina.* Journal of Complementary and Integrative Medicine, 5(1).
Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, Hu ZX, Xiao JZ, Cao HB, Liu PA, Jiang XG (1997). Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance: the Da Qing IGT and Diabetes Study. Diabetes care, 20(4):537-544.
Pignone M, Alberts MJ, Colwell JA, Cushman M, Inzucchi SE, Mukherjee D, Rosenson RS, Williams CD, Wilson PW, Kirkman MS (2010). "Aspirin for primary prevention of cardiovascular events in people with diabety of a position statement of the American Diabetes Association, a scientific Journal of the American Heart Association, and an expert consensus document of the American College of Cardiology Foundation. Circulation, 121(24):2694-2701.
Rader DJ (2007). Effect of insulin resistance, dyslipidemia, and intra–abdominal adiposity on the development of cardiovascular disease and diabetes mellitus. The American journal of medicine, 120(3):S12-S18.
Rates SM (2001). Plant as source of drug. Toxicon. 39(5):603-613.
Robertson RP (1995). "Antagonist: diabetes and insulin resistance–philosophy, science, and the multiplier hypothesis. The Journal of Laboratory and Clinical Medicine, 125(5):560-564.
Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. Nature, 414(6865):799-806.
Tiwari AK, Rao JM (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Current science, 83(1):30-37.
Torel J, Cillard J, Cillard P (1986). Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry, 25:383-385.
Ugwu CE, Ejike CC, Alumana EO, Ezeanyika LUS (2010).Effect of dietary incorporation of Gongronema latifolium, Vernonia amygdalina and telfairia occidentalis leaves at various levels on the lipid profile of rats Indian Journal of Animal Nutrition, 27(3):303-308.
UKPDS (UK Prospective Diabetes Study Group), Intensive blood glucose with sulphonyl areas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (UKPDS 33). Lancet, 353:837-853.
Van–Burden T, Robinson W (1981). Formation of complexes between protein and Tannin acid. Journal of Agricultural and Food Chemistry, 1:77.
World Health Organisation (1993). Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 28:13-14.

Yokozawa T, Cho EJ, Sasaki S, Satoh A, Okamoto T, Sei Y (2006). The protective role of Chinese prescription Kangen–karyu extract on diet–induced hypercholesterolemia in rats. Biological and Pharmaceutical Bulletin, 29(4):760-765.

Zheng J, Yang B, Tuomasjukka S, Ou S, Kallio H (2009). Effects of latitude and weather conditions on contents of sugars, fruit acids, and ascorbic acid in black currant (*Ribes nigrum* L.) juice. Journal of agricultural and food chemistry, 57:2977-2987.

Vol. 12(19), pp. 240-247, 8 June, 2018
DOI: 10.5897/AJPP2017.4777
Article Number: 64A963457513
ISSN: 1996-0816

http://www.academicjournals.org/AJPP

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Full Length Research Paper

Neurohistological and immunohistochemical effects of prophylactic ethanolic leaf extract of *Nauclea latifolia* and Artemether/Lumefantrine on the hippocampus of *Plasmodium berghei*-infected mice

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Received 5 April, 2017: Accepted 23 April, 2017

The prophylactic effect of ethanolic leaf extract of *Nauclea latifolia*, widely used in herbal malarial treatment, was compared against standard drug Coartem® (Artemether/Lumefantrine) in *Plasmodium*-infected mice was investigated, and thereafter analysed for organosomatic index, parasitemia, histomorphological and immunohistochemical changes in the hippocampus. Twenty male mice about 6 to 8 weeks weighing 20 to 24 g were alloted into four groups of five mice each. Group 1 served as control received placebo; group 2 received extract 500 mg/kg; group 3 received extract 1000 mg/kg; and group 4 received 5 mg/kg of Artemether/Lumefantrine. Extract and drug administrations were performed for 3 days, and thereafter mice were infected with 10^6 of *Plasmodium berghei* parasites, and monitored for 4 days, after which the experiment was terminated. Thick blood smear were prepared from lateral tail vein, then under anesthesia via a cocktail of xylaxine and ketamine, intracardiac perfusion was performed first with phosphate buffered saline to clear systemic blood, and then 4% paraformaldehyde to fixed brain for routine histology and immunohistochemistry. Result indicates organosomatic index was not statistically significant, parasitemia in the treated groups were significantly ($p<0.05$) decreased compared to control and were corroborated in the photomicrographs of respective blood morphology, however, histologically there was moderate to severe distortion of the hippocampus across the groups, but glial fibrillary acidic protein expression, a marker for neurotoxicity indicated that group 4 had the most immunolabelling intensity compared to other test groups. In conclusion, the prophylactic ethanolic extract of *N. latifolia* and Artemether/Lumefantrine cleared parasitemia also seen in the blood morphology, and low dose N. latifolia plausibly has better safety and hippocampal toxicity profile with decreased neuronal shrinkage and distortions, with a more down regulated glial fibrillary acid protein than higher extract doses and Artemether/lumefantrine.

Key words: Malaria, hippocampus, neuronal perturbation, Artemether/Lumefantrine, *Nauclea latifolia*.

INTRODUCTION

The staggering number of malaria cases and mortality from malaria infection requires consistent efforts at actionable plans to mitigate the trajectory, as according to World Health Organization (WHO) Malaria Report (2015)

the global estimated number of malaria cases was 214 million, and the number of deaths was about 438000, and percentage-wise the WHO African Region had the most number of cases at 88% and the most number of deaths at 90%, far higher than other WHO regions and this has remained so for several decades now. The current practice in treating malaria is based on the concept of combination therapy, and Artemether/Lumefantrine is the drug of choice (WHO, 2010). Effects of drugs on biochemical targets possibly precede manifestation of morphologic endpoints as chemically induced neurodegeneration is evident by different patterns of neuronal cell death, gliosis, swollen or destroyed axons, or destruction of the myelin sheath. *In vitro* studies of brain stem cells show that they were selectively sensitive against artemisinin, in contrast to cells from other brain regions, such as the cortex (Schmuck et al., 2002).

Researchers with interest in natural products have intensified their efforts toward scientific evaluation of traditional medicines (Taheri et al., 2012), and numerous claims abound on the potency and the use of these plants which require further authentication to establish their scientific bases and efficacy especially in the management of certain diseases in rural communities (Builders et al., 2012). Some ethnic tribes in Akwa Ibom State, Nigeria, drink the leaf decoction of *Nauclea latifolia* (NL) for the prevention and cure of malaria (Udobre et al., 2013). *N. latifolia* have been reported to possess antiplasmodial activity (Ajaiyeoba et al., 2004; Asase et al., 2005; Adzu et al., 2013). Still little research data exist on the neurotoxicity of these antimalarial bio-chemicals *in vivo* studies especially their prophylactic impact.

Neuronal cell damage in the sector CA1 of the hippocampus is common in severe and cerebral malaria escially brain-derived neurotrophic factor (BDNF) levels in the hippocampus relate with memory impairment (Comim et al., 2012; Kihara et al., 2009). This study was to investigate the neurohistomorphological effects of prophylactic ethanolic leaf extract of *N. latifolia* compared with Arthemeter-Lumefantrine on the hippocampus, blood morphology and organo-somatic index of *Plasmodium berghei* infected Swiss albino mice (*Mus musculus*).

MATERIALS AND METHODS

Experimental animals

Twenty (20) male Swiss albino mice were obtained weighing 20 to 24 g from the animal house of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. The animals were acclimatized for two weeks before the start of the investigation at the institution's animal holding room, in well ventilated mice cages and maintained under controlled environmental conditions of temperature 25 ± 5°C and 12 h light/dark cycle. All the animals were allowed access to feed (rat mash; Vital Feeds from Grand Cereals Limited, Jos, Plateau State), and water *ad libitum*. All procedures involving animals in this study conformed to the guide for the care and use of laboratory animals (National Institute of Health, 2011) and granted approval by the Department of Anatomy ethical committee, University of Uyo.

Collection and authentication of plant sample

Fresh leaves of *N. latifolia* were harvested from the medicinal farm of Pharmacology and Toxicology Department, University of Uyo were identified and authenticated by the Curator at the Herbarium of Department of Pharmacology and Toxicology, University of Uyo with specimen and voucher number UUH/67 (g) deposited.

Plant extraction

Fresh leaves of *N. latifolia* macerated in 95% ethanol (Sigma Aldrich St Louis USA) in a flat bottom flask and kept for 72 h at room temperature. The macerated leaves were filtered and the filtrate concentrated in water-bath at 45°C to dryness. Extract was weighed and stored in the refrigerator until required for use.

Evaluation of prophylactic activities

The repository activity of the extract and Coartem® (Artemether/Lumefantrine) was assessed by using the method described by Peters (1965). The mice were randomly divided into four groups of five mice each. Group 1 served as the negative control and received 10 ml/kg of normal saline (0.9% Nacl), group 2 received 500 mg/kg of ethanolic leaf extract, group 3 received 1000 mg/kg of ethanolic leaf extract, and group 4 received 5 mg/kg of Coartem®. Administration of the extract and drug was for three consecutive days (D0 - D2). On the fourth day (D3) the mice were inoculated with *P. berghei berghei.* The parasitaemia level was assessed by blood smears 72 h later.

Parasite inoculation

P. berghei was obtained commercially from National Institute of Medical Research (NIMER), Yaba, Lagos Nigeria in three host mice, and each mouse was inoculated intraperitoneally with 0.2 ml of infected blood containing about 10^6 *P. berghei* parasitized erythrocytes. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (Odetola and Basir, 1980).

Experimental treatments

Coartem® Novartis a popular ACT purchased from a reputable pharmacy within the Uyo metropolis was dissolved in distilled water and treatment performed according to method by Olorunnisola and Afolayanin (2011). *N. latifolia* ethanolic leaf extract was dissolved in 20% Tween® 80 and administered orally based on body weights and in accordance to 10 and 20% of LD_{50} for low and medium

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Table 1. Prophylactic effect of Artemether-Lumefantrine and *Nauclea latifolia* onorganosomatic index in *Plasmodium*-infected mice.

Treatment groups (n=5)	Brain weight (g)	Initial body weight (g)	Final body weight (g)	Change in body weight (%)	Organo-somatic index
Distilled water - 10 ml/kg	0.36±0.01	21.40±0.87	20.60±0.75	-3.74	1.74±0.05
Ethanolic extract 500 mg/kg	0.41±0.01^{NS}	23.00±0.71	22.80±0.58^{NS}	-0.87	1.84±0.09^{NS}
Ethanolic extract 1000 mg/kg	0.40±0.01^{NS}	24.60±0.51	26.60±0.40*	+8.13	1.52±0.06^{NS}
Coartem® - 5 mg/kg	0.42±0.00^{NS}	23.20±0.66	24.20±1.11^{NS}	+4.31	1.66±0.09^{NS}

Coartem® - Artemether-lumefantrine; Values are expressed as Mean ± SEM; n=5; NS: Not significant compared with control; *p<0.05 compared with control.

Table 2. Prophylactic effect of Artemether-lumefantrine and *N. latifolia* on parasitemia in *P. berghei*-infected mice.

Treatment groups (n=5)	Parasite density (Mean±SEM)	Chemosuppression (%)
Distilled water - 10 ml/kg	280.80±51.43	-
Ethanolic extract 500 mg/kg	110.00±26.14***	60.61
Ethanolic extract 1000 mg/kg	57.00±21.45***	79.69
Coartem® 5 mg/kg	0.00±0.00***	100.00

Coartem® - artemether-lumefantrine; ***p < 0.001 compared with control.

dosage, respectively. Meanwhile, Giemsa stained blood smears obtained from the tail vein of the mice was made and viewed under oil immersion at ×100 magnification at end of experiment to determine parasitemia by direct enumeration (WHO, 2000) and images were obtained with a digital microscopic camera.

Animal sacrifice and tissue processing

Xylazine and ketamine cocktail was injected to mice at 0.2 ml, when completely unconscious, alcohol pad was used to sterilize the trunks, mid line insertion performed, thereafter intracranial perfusion via cardiac puncture with buffered saline to flush out the blood for 1 min via left ventricle, and right atrium pierced to release circulating fluid, and then buffered formalin was released via a drip-set controlled by a valve until mice tail was stiff, and then brains dissected out from the skull, dried on a filter paper, weighed and fixed in 4% paraformaldehyde for immunohistochemistry. Plastic embedded brain sections were also processed for light microscopy by method as described (Cardiff et al., 2014). The immunolabelling of glial fibrillary acidic protein (GFAP) according to method described (Faddis and Vijayan, 1988; O'Callaghan and Sairam, 2005) was cut at 5 microns thick. Sections were allowed to heat on hot plate for 1 h, then transferred to xylene, alcohols and water respectively. Antigen retrieval method was performed using citric acid solution pH 6.0 in a pressure cooker for 15 min. Sections were exposed to running tap water for 3 min. Peroxidise blocker was used on the sections for 15 min and then washed for 2 min with phosphate buffered saline (PBS) with tween 20. Protein blocker was carried out with Novocastra® protein block for 15 min and then washed with PBS for 2 min, and incubated with primary antibody, monoclonal mouse anti-glial fibrillary acidic protein (GFAP) DAKO 1 in 100 dilution for 45 min, washed in PBS for 3 min, and added rabbit anti-mouse secondary antibody for 15 min all at room temperature. Tissue section was then washed twice with PBS. Polymer was thereafter added and allowed for 15 min, washed twice with PBS and then added the diaminobenzidine (DAB) chromogen diluted 1 in 100 with the DAB substrate for 15 minutes, and washed with water, and counterstained with haematoxylin for 2 min. Tissue section were washed again, dehydrated, cleared and mounted in DPX mountant.

Statistical analysis

Data obtained in the study were expressed as mean ± standard error of mean (SEM) and analyzed using one-way analysis of variance (ANOVA) to determine the difference between the experimental groups and the control group, and the post-hoc test (Student-Newman Keuls) for comparison and values was regarded as significant at ($p<0.05$).

RESULTS

Table 1 shows that brain weights of test groups were not significantly changed compared control, the final body weight of the ethanolic extract of NL was significantly increased compared to the control, but the organosomatic index of tests groups compared to control was significantly unchanged. Table 2 shows a statistically significant decrease in the parasite density of the ethanolic extract groups compared to control at a dose dependent level, and Artemether-Lumefantrine proved to possess the most of chemosuppression, and these is evident in the micrographs of the blood morphology in Figure 1A to D. Figures 2A to D is the histomorphology of the hippocampus; Figure 2A shows partially dark stained neurons with hypochromatic parenchyma (inference – severely affected); Figure 2B shows neuronal hypertrophy with disperse neuronal shrinkage (inference – severely affected); Figure 2C shows prominent neuronal atrophy, polymorphic neurons with few foci pyknosis (inference – severely affected); 2D has a

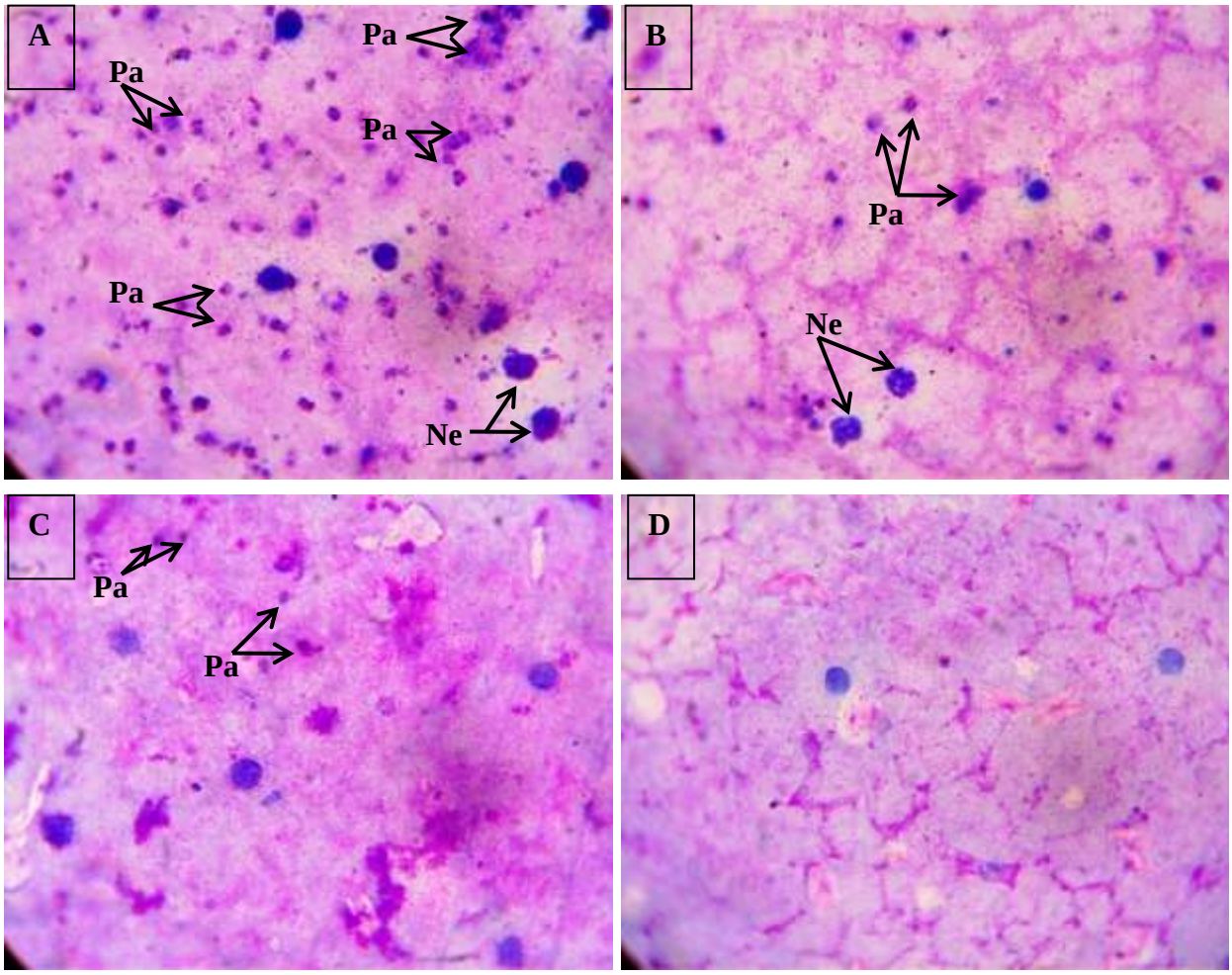

Figure 1. (A-D) Prophylactic effect of ethanolic leaf extract of *Nauclea latifolia* and 5 mg Artemether-Lumefantrine per kg body weight of *Plasmodium* infected mice on blood morphology.

reduced cellular layer with few atrophic neurons and dispersed vacuolations (inference – moderately affected). Immunohistochemical expression of glial fibrillary acidic protein as shown in Figure 3A to D; groups 1, 3 and 4 indicate severe immunoreactivity with the exception of group 2 which had moderate immunolabelling intensity, however group 4 (Figure 3D) had the most severe neurotoxic presentation.

DISCUSSION

Acute toxicity study of the ethanolic leaf extract of *N. latifolia* did not cause mortality at up to 5000 mg/kg body weight of mice, and hence can be claimed to be relatively safe (Gidado et al., 2011). The organosomatic indices (OSI) of this prophylactic study in Table 1 was not significantly ($p>0.05$) changed when treated groups (2 to 4) compared to the control (infected untreated). This OSI outcome may not be unconnected with the sub-acute duration of the experiment. However, final body weights was significantly increased ($p<0.05$) when ethanolic leaf extract 1000 mg/kg group was compared with all the groups. A dose-dependent reduction in body weights of rats administered root extract of *N. latifolia* compared with the control group has previously been reported (Odey et al., 2012). Organosomatic indices are described as the ratios of organ to body weights; measured organ in relation to body mass can be directly linked to toxic effects of chemical on target organ (Giulio and Hinton, 2008).

Plants that contain bioactive compounds like alkaloids, flavonoids and triterpenoids may in part contribute to their plasmocidal activity and therefore explain their mechanism of action (Okokon et al., 2012). Curative malaria studies have shown evidence that aqueous leaf extract *N. latifolia* treatment at 200 and 300 mg/kg body weight respectively eliminated the *P. berghei* parasites and protected against oxidative damage in liver and brain tissues even better than chloroquine (Onyesom et al.,

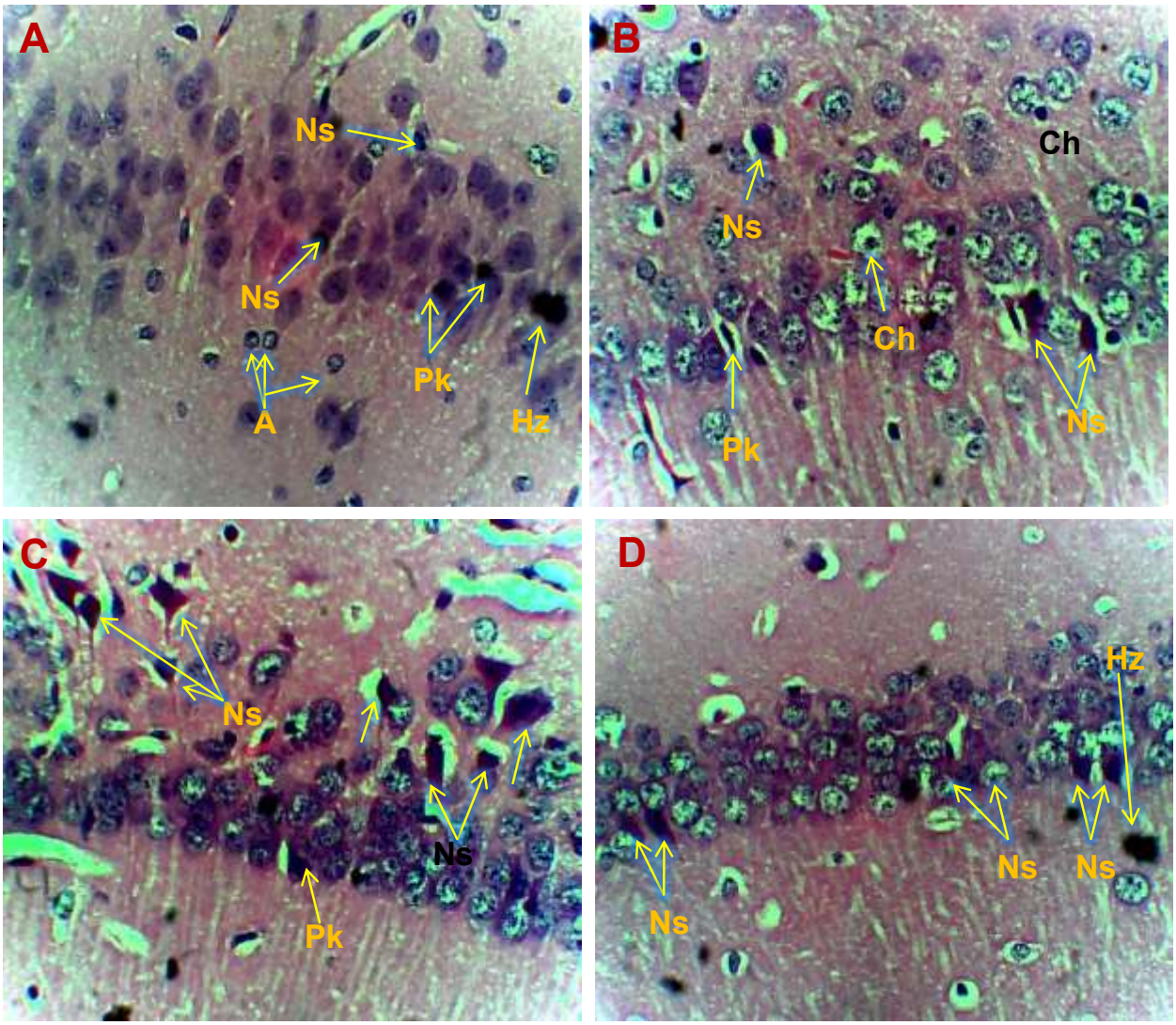

Figure 2. (A-D) Prophylactic effect of ethanolic leaf extract *of Nauclea latifolia* and Artemether/Lumefantrine on hippocampal histomorphology in *Plasmodium*-infected mice. PK: Pyknosis; Ns: neuronal shrinkage; Hz: hemozoin; A: astrocytes.

2015), while ethanolic leaf extract of *N. latifolia* via its phytochemical bioavailability decreases *P. berghei* in dose dependent manner, offers moderate neuro-protection to hippocampus of infected mice, when compared to hippocampal H&E findings of 5 mg/kg Artemisinin/Lumefantrine treated group which showed severely distorted neurons (Edagha et al., 2017), but mildly expressed GFAP, a down-regulating marker of the neuroinflammatory protein which may suggest GFAP level declined prior to slightly improved neuronal morphology seen in the respective H&E group, whereas in this prophylactic study the reverse is now reported, in which hippocampal H&E showed in Figure 2D that 5 mg/kg Artemisinin/Lumefantrine presented mild neuronal distortion, but a moderately expressed GFAP suggestive of an underlying trauma and/or infection, perhaps inducing an immunologic response required to overcome the circulating parasite.

Nucleic acid base pairing of *Plasmodium* parasite chelate with flavonoids (Lui et al., 1992), and triterpenes like quassinoids are potent protein inhibitors (Liao et al., 1976). Oxidative damage induced by the malarial parasite is ameliorated by this bioactive activity (Alli et al., 2011). Parasitemia outcome following prophylactic activity as shown in Table 2 indicated significantly ($p<0.001$) lower parasite density and percentage chemosuppression in the treated groups compared to the control, suggesting that the antiplasmodial activity of *N. latifolia* ethanolic leaf extract is based on the antioxidant and plasmocidal effects of its bioavailable phytochemicals (Alli et al., 2011). A dose dependent antiplasmodial activitiy in the stem-bark of aqueous and fractions of *N. latifolia* extracts has been reported to be based on their phytochemicals (Ettebong et al., 2015).

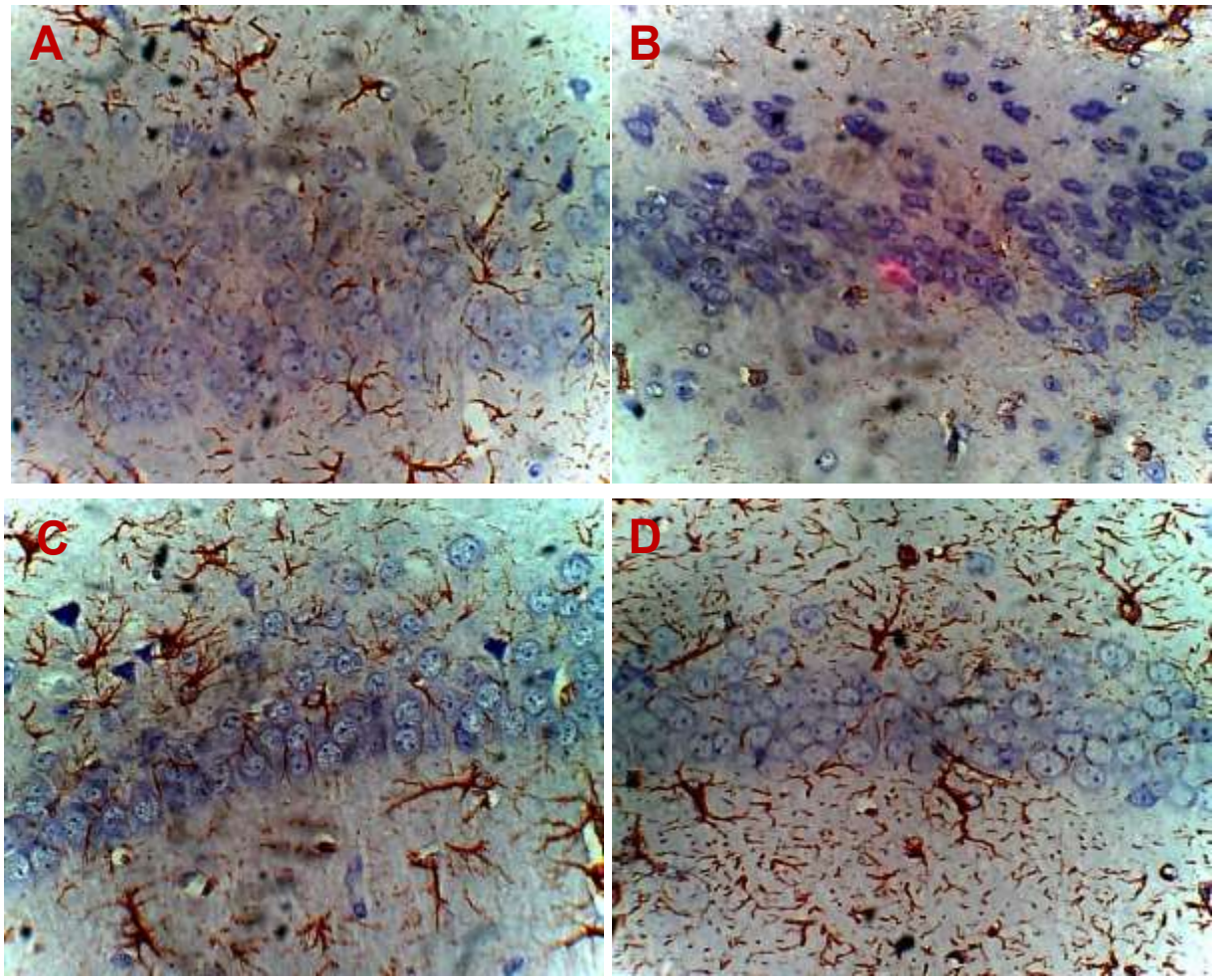

Figure 3. (A-D) Prophylactic effect of ethanolic leaf extract *of Nauclea latifolia* and Artemether/Lumefantrine on hippocampal glial fibrillary acidic protein expression in *Plasmodium* infected mice.

Photomicrographs of the thick blood smears as shown in Figure 1A to D corroborates the antiplasmodial activity of the plant extract.

Neurotoxicity is the study of the undesirable consequences that develop in the central nervous system or peripheral nervous system or both after an organism is exposed to a neurotoxic agent during development or adulthood (Bolon and Graham, 2011), and can present as aberrations in neural structure (toxicological neuropathology) or function (including altered behaviour, biochemistry, cognition, or impulse conduction), or both (Bolon and Graham, 2011). Haematoxylin and eosin (H&E) is a cell body-specific stain and it is quite valid to reach a conclusion of neurotoxicity when using such staining tool, (Switzer and Butt, 2011). Results from the H&E staining of the hippocampal neuronal cell bodies presented for the prophylactic activity as shown in Figure 2A indicates severe cellular distortion of the hippocampal architecture; Figure 2B had moderate morphological changes with inflammation and neuronal shrinkages being common and neuronal shrinkage is an early and easily recognizable indicator of neuronal degeneration in the hippocampus (Bonde et al., 2002). The large pyramidal cells in area CA1 are exceptionally sensitive to oxygen deprivation and die after few minutes without a supply of fresh arterial blood, and pathologists call the area CA1, Sommer's sector. The hippocampal pyramidal cells are among the first to be affected in a variety of conditions that lead to loss of memory and intellectual functions (Kiernan, 2009). The hippocampus as shown in Figure 2D showed depletion of pyramidal cells (lower neuronal density). The low dose of the ethanolic extract at 500 mg/kg was perhaps not sufficiently capable of ameliorating the neurotoxic effect of the *P. berghei*. Although at 1000 mg/kg of the extract of *N. latifolia* moderately affected the hippocampus with the intact cell layers indicating an agonistic effect perhaps due to the prophylactic protection of the extracts against the neurotoxic *P. berghei* via a dose dependent antioxidant constituents in the treatment. Antioxidant nutrients of

plants have the potential to scavenge for free radicals in the system and neutralize them before they do any damage to cells. *N. latifolia* could be a potential source of pharmacologically active natural products and/or for development of neutraceuticals (Egbung et al., 2013), especially from the rich source of flavonoids, alkaloids and tannins. The result of glial fibrillary acidic protein (GFAP)as shown in Figure 3A to D revealed moderate to severe immunoreactive astrogliosis. GFAP expression can be regarded as a sensitive and reliable marker that labels most, if not all, reactive astrocytes that are responding to CNS injuries (Sofroniew and Vinters, 2010) and reactive astrogliosis is prominent in most CNS infections. Since the hippocampus is the principal brain structure for learning and memory acquisition, GFAP up-regulation may support hypothesis of learning and memory impairment.

In this study, prophylactic Artemether-Lumefantrine ensured a more significant decline in parasitemia load compared with dose dependent ethanolic leaf extract of *N. latifolia*, although Artemether/Lumefantrine treated group showed a moderate hippocampal GFAP immunopositivity and neuronal distortion mainly shrinkage with presence of hemozoin in the hippocampal CA1 region in *Plasmodium* infected mice stained with H&E similar with extract treated groups, so the prophylactic herbal or Artemether/Lumefantrine clears parasitemia but only mildly protects the hippocampus at higher extract doses after *P. berghei* infection in mice.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge Mr. Malachy Nsikan, Department of Pharmacology and Toxicology, University of Uyo, Uyo, Nigeria and Mr. Jonathan Madukwe, Department of Histopathology, National Hospital Abuja for their technical assistance.

REFERENCES

Adzu B, Mustapha KB, Masimirembwa C, Obodozie O, Kirim RA, Gamaniel KS (2013). Simulation of metabolism based herb drug interaction: Towards safe and efficacious use of NIPRD-AM1. Avicenna J. Phytomed. 3(3):201-204.

Ajaiyeoba E, Ashidi J, Abiodun O, Okpako L, Ogbole O, Akinboye D, Falade C, Bolaji O, Gbotosho G, Falade M, Itiola O, Houghton P, Wright C, Oduola A (2004). Antimalarial ethnobotany: In vitro antiplasmodial activity of seven plants identified in the Nigerian middle belt. Pharm. Biol. 42(8):588-591.

Alli LA, Adesokan AA, Salawu OA, Akanji MA, Tijani AY (2011). Antiplasmodial activity of aqueous root extract of *Acacia nitotica*. Afr. J. Biochem. Res. 5(7):214-219.

Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MSJ (2005). Ethnobotanical study of some Ghanaian anti-malarial plants. J. Ethnopharmacol. 99:273-279.

Bolon B, Graham DG (2011). Fundamental Neuropathology for Pathologists: An introduction. 1st ed., New Jersey: John Wiley and Sons, P 3.

Bonde C, Noraberg J, Zimmer J (2002). Nuclear shrinkage and other markers of neuronal cell death after oxygen-glucose deprivation in rat hippocampal slice cultures. Neurosc. Lett. 327:49-52.

Builders MI, Isichie CO, Aguiyi JC (2012). Toxicity studies of the extracts of *Parkia biglobosa* stem bark in rats. Br. J. Pharm. Res. 2(1):1-16.

Cardiff RD, Miller CH, Munn RJ (2014). Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harbor protocols. 2014:6.

Comim CM, Reis PA, Frutuoso VS, Fries GR, Fraga DB, Kapczinski F, Zugno AI, Barichello T, Quevedo J, Castro-Faria-Neto HC (2012). Effects of experimental cerebral malaria in memory, brain-derived neurotrophic factor and acetylcholinesterase activity in the hippocampus of survivor mice. Neurosc. Lett. 523:104-107.

Edagha IA, Peter AI, Aquaisua AN (2017). Histopathological effect of *Nauclea latifolia* ethanolic leaf extract and artemether/lumefantrine on the hippocampus of *P. berghei*-infected mice. Interna. J. Brain Cogni. Sci. 6(1):9-16.

Egbung GE, Atangwho IJ, Iwara IA, Odey MO, Ebong PE (2013). Chemical composition of root and stem bark extracts of *Nauclea latifolia*. Arch. Appl. Sci. Res. 5(3):193-196.

Ettebong EO, Edwin UPM, Edet EC, Samuel EU, Ezekiel AO, Dornu TV. (2015) In vivo antiplasmodial activities of Nauclea latifolia. Asian J. Med. Sci. 6:(3)6-11

Faddis BT, Vijayan VK (1988). Application of glial fibrillary acidic protein immunohistochemistry in quantification of astrocytes in the rat brain. Am. J. Anat. 183:316-322.

Gidado A, Ameh DA, Atawodi SE, Ibrahim S (2011). Evaluation of the acute and subchronic toxicity of *Nauclea latifolia* leaf ethanolic extract in rats. In: Proceedings of the 31st annual conference of the Nigerian society of biochemistry and molecular biology, p. 151.

Giulio RT, Hinton DE (2008). Toxicology of Fishes. CRC Press, Taylor and Francis Group, Boca Paton. P 1071.

Kiernan JA (2009). Limbic system: The hippocampus and amygdala. In: *Barr's the human nervous system an anatomical viewpoint* 9th ed., Baltimore: Lippincott Williams and Wilkins. pp. 268-274.

Kihara M, Carter JA, Holding PA, Vargha-Khadem F, Scott RC, Idro R, Fegan GW, de Haan M, Neville BGR, Newto CRJC (2009). Impaired everyday memory associated with encephalopathy of severe malaria the role of seizures and hippocampal damage. Malar. J. 8:273.

Liao YF, Kupchan SM, Horwitz SB (1976). Mode of action of anti-tumour compound bruceantin an inhibitor of protein synthesis. Mol. Pharmacol. 12:167-176.

Lui KC, Yang SC, Roberts MF (1992). Antimalarial activity of *artemisia annua* flavonoids from whole plants and cell cultures. Plants Cell. 2:637-640.

National Institute of Health (2011). Guide for the care and use of laboratory animals, 8th edition, Washington (DC): National Academies Press (US) pp 1-217.

O'Callaghan J, Sairam K (2005). Glial fibrillary acidic protein and related glial proteins as biomakers of neurotoxicity. Expert Opinion Drug Safety. 4:433-442.

Odetola A, Basir O (1980). Evaluation of antimalarial properties of some Nigerian medicinal plants. In: Sofowora, A. (Ed.), In: Proceedings of African bioscience network, federal ministry of science and technology, Nigerian society of pharmacology and drug research and production unit, University of Ife organized workshop, Ife. pp. 275-283.

Odey MO, Gauje B, Udiba UU, Johnson JT, Inekwe VU, Adegbe EA (2012). Effect of activity directed administration of ethanolic extracts of *Nauclea latifolia o*n the body and organ weights of hypertensive induced albino Wistar rats. Ann. Biol. Res. 3(11):5114-5118.

Okokon JE, Ndehekedehe I, Akpan EJ (2012). In vivo antiplasmodial and antipyretic activities of *Smilax krausiana.* Phytopharmacology 3(2):376-385.

Olorunnisola OS, Afolayanin AJ (2011). In vivo anti-malaria activity of methanolic leaf and root extracts of *Sphenocentrum jollyanum Pierre*.

Afr. J. Pharm. Pharmacol. 5(14):1669-1673.
Onyesom I, Osioma E, Okereke PC (2015) Nauclea latifolia aqueous leaf extract eliminates hepatic and cerebral Plasmodium berghei parasite in experimental mice. Asian Pac. J. Trop. Biomed. 5(7): 546–551.
Peters W (1965). Drug resistance in *Plasmodium berghei*. Exp. Parasitol. 17:80-89.
Schmuck G, Roehrdanz E, Haynes RK, Kahl R (2002). Neurotoxic mode of action of artemisinin. Antimicrob. Agents Chemother. 46(3):821-827.
Sofroniew MV, Vinters HV (2010). Astrocytes: Biology and Pathology. Acta Neuropathol. 119:7-35.
Switzer RC, Butt MT (2011). Histological markers of neurotoxicity (Nonfluorescent). In: Fundamental Neuropathology for Pathologists and Toxicologists. Principles and Techniques Bolon B, Butt MT (Eds.), A John Wiley and Sons Publication, New Jersey. P 181.
Taheri S, Zarei A, Ashtiyani SC, Rezaei A, Zaheiri S (2012). Evaluation of the effects of hydroalcoholic extract of *Berberis vulgaris* roots on the activity of liver enzymes in male hypercholestrolaemic rats. Avicenna J. Phytomed. 2(3):153-161.
Udobre AS, Udobang JA, Udoh AE, Anah VU, Akpan AE, Charles GE (2013). Effect of methanol leaf extract of *Nauclea latifolia* on albino mice infected with *Plasmodium berghei berghei.* Afr. J. Pharmacol. Ther. 2(3):83-87.
World Health Organization (WHO) (2015). World malaria report, Geneva, Switzerland. P 44.
World Health Organization (WHO) (2010). Guidelines for the treatment of malaria, Second Edition. pp 1-194.
World Health Organization (WHO) (2000). Bench aids for the diagnosis of malaria infections. pp. 1-24.

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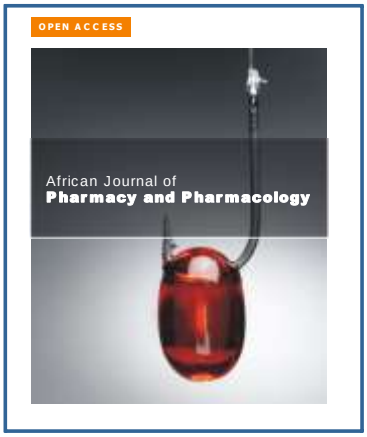

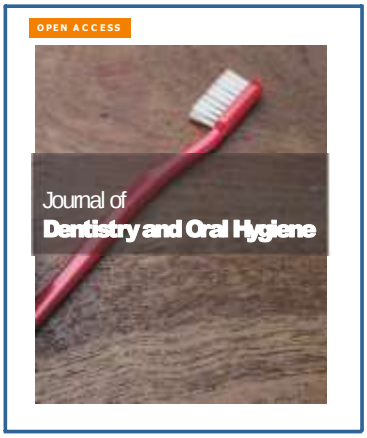

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